LIPID CHANGES IN GERMINATING *TARAMIRA* (*ERUCA SATIVA*) AND LINSEED (*LINUM USITATISSIMUM*)

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ABSTRACT

The changes in the total oil, fatty-acid composition and the lipid composition at different stages of the germination of *taramira* and linseed were studied. During germination, the triglycerides are degraded, and oleic, linoleic and linolenic acids are utilized to a greater extent in the *taramira* seeds. Erucic acid is preferentially incorporated into the newly synthesized triglycerides. In linseed, the breakdown of triglycerides in the initial stages is very slow. Afterwards, however, the glycerides are rapidly degraded, and free fatty acids are liberated during germination.

LIPID changes during the germination of oilseeds have so far been confined to the determination of iodine value, acid value and oil content. Changes in the total oil content and the acid value of germinating *taramira* seeds were studied by Kartha (1961). Zimmerman & Klosterman (1965) for the first time fractionated the lipids of the germinating seeds of linseed and determined the composition of fatty acids of different lipid fractions by using gas liquid chromatography (G.L.C.). We have studied the lipid changes in the cotyledons and hypocotyls of the germinating seeds of *taramira* and linseed by fractionating the lipids into partial glycerides, free sterols, free fatty acids and triglycerides and by determining the fatty-acid composition of these fractions by using G.L.C. and the results are presented in this paper.

MATERIAL AND METHODS

Seed Germination

In *taramira*, germination was secured under aerobic conditions. The seeds of two varieties (Local and Selection A) were sown in enamelled trays (24"×12"), each containing 1 kg. of sulphuric-acid-washed sand. The weighed amount of *taramira* seed (50 g.) was spread on the sand in each tray and the requisite amount of water was applied. After every 24 hours, distilled water was applied to every tray in equal quantity. At 24-hour intervals, the germinating seeds (seedlings) were removed and washed with distilled water. The hypocotyls and cotyledons were separated with a pair of scissors and fat was extracted from them on the same day. The germination of linseed was carried out at 37°C. The seeds (5 g.) were placed in 14-cm. petri-dishes, containing filter-paper wetted with 4 ml. of distilled water. The petri-dishes were placed in an incubator at 37°C. in the dark. Two ml. of water was added to each dish at 18-hour intervals. The germinating seeds of one petri-dish were removed, washed with distilled water and fat was extracted from them at each 18-hour interval. This study was continued up to 90 hours after the sowing of the seeds.

The total lipids were extracted by using the method of Folch *et al.* (1957). Separation into total polar and non-polar lipids was achieved by the silicic acid-column chroma-

tograpgy, according to Pomeranz *et al.* (1966). The non-polar lipids were further fractionated on thin layer chromatography (TLC) according to Malins & Mangold (1960), and identified by comparing with standards, i.e. tripalmitin, cholestrol, palmitic acid and cholestrol acetate. Free fatty acids were detected with blue colour under ultraviolet light after the Lieberman Burchard reaction. The quantitative evaluation of individual lipids was done by using the densitometric method of Privett *et al.* (1965). The spot due to sterol esters and hydrocarbons emerged with the non-polar pigments, and thus, this lipid was not taken into consideration. It was assumed that the non-polar oil consisted of only partial glycerides, free sterols, free fatty acids and triglycerides. Then the relative percentage of each individual constituent was converted into g./100g. of cotyledons as follows :

g. of lipid/100g. cotyledons = % of the lipid × oil per 100 g. of green seedlings

The methyl esters were prepared by using the method of Instrument Technique Committee (1966). The fractionation of the methyl esters was done, using an Aerograph HYFI (Model 600 C) through a stainless-steel column 6 metres × 4.11 mm., packed with 20 per cent diethylene glycol succinate (DEGS) on a 60-80 mesh chromosorb W. Tentative identification of the peaks was done by comparing their retention time with the retention times of the standard known fatty acids. Areas under the peaks were calculated with a planimeter and converted into direct relative percentages.

RESULTS AND DISCUSSION

Lipid Changes in *Taramira* (Cotyledon)

A gradual decrease in the concentration of oil and the triglyceride fraction was observed during the whole germination period, except during the first 24 hours (Table 1). In contrast, there was a little increase in the concentration of free fatty acids and the partial glycerides in the seedlings. Robeiz *et al.* (1965) have suggested that the mitochondria function properly only after the germination has proceeded for a critical minimum period. As mitochondria are responsible for the mobility of fat, no change of oil content during the early stages of germination is to be expected on this ground. There was no accumulation of free fatty acids and partial glycerides during germination, because they are actively converted into carbohydrates (Kornberg & Beevers, 1957).

TABLE 1

Non-polar lipid composition (on relative basis) of *taramira* seed during germination (g./100 g. cotyledons)

Particulars	Days after germination						
	1	2	3	4	5	6	7
			(a) *Taramira Local*				
g. oil in 100 g. seedlings	35.00	32.00	18.00	14.00	11.00	7.00	6.00
Partial glycerides	0.81	0.77	0.58	0.41	0.43	0.22	0.26
Free sterols	0.49	0.45	0.47	0.48	0.35	0.09	0.23
Free fatty acids	0.98	1.02	0.61	0.48	0.75	0.72	0.85
Triglycerides	32.80	29.76	16.34	12.64	9.54	5.97	4.66
			(b) *Taramira Selection A*				
g. oil in 100 g. seedlings	35.30	32.00	24.00	18.00	14.00	9.00	8.00
Partial glycerides	0.60	0.60	0.58	1.33	1.43	1.01	0.78
Free sterols	0.20	1.66	1.20	0.83	1.34	0.48	0.57
Free fatty acids	1.17	0.99	1.08	0.97	1.09	1.06	1.47
Triglycerides	32.33	28.75	21.22	14.87	10.14	5.46	5.18

TABLE 2

Non-polar lipid composition (on relative basis) of linseed during germination (g. 100 g./cotyledons) under anaerobic conditions (Densiometric determination)

Particulars	Hours after germination					
	1	18	36	54	72	90
	(a) *Linseed K_2*					
Oil percentage (on dry-matter basis)	40.60	38.00	34.00	26.00	20.00	16.00
Partial glycerides	0.61	2.43	1.77	0.57	0.64	0.54
Free sterols	0.49	0.87	1.67	1.61	1.36	1.91
Free fatty acids	0.44	2.28	3.74	3.85	3.20	6.14
Hydrocarbons+sterol esters	1.58	1.92	1.05	0.31	0.36	0.67
Triglycerides	36.62	30.89	25.12	18.51	13.52	6.88
	(b) *Linseed Local*					
Oil percentage (on dry-matter basis)	39.40	37.00	31.00	16.00	18.00	16.80
Partial glycerides	0.95	1.63	0.99	0.73	0.58	0.60
Free sterols	0.96	0.89	1.61	2.34	1.84	2.09
Free fatty acids	0.87	3.11	4.41	4.42	3.67	6.17
Hydrocarbons+sterol esters	1.58	1.92	1.05	0.31	0.36	0.67
Triglycerides	35.14	29.45	22.94	18.20	11.55	7.27

The changes in the concentration of fatty acids in the oil of germinating *taramira* seeds indicate a non-selective utilization of the individual fatty acids (Table 3). However, a selective accumulation of erucic acid was observed in the oil of the cotylendons. In contrast to its increased concentration in the oil of the cotyledons, the concentration of erucic acid was practically constant in the oil of the hypocotyl. It is possible that other unsaturated fatty acids (linoleic and linolenic) were converted into erucic acid through the mediation of some unknown chain-elongation enzymes, as suggested by Stumpf (1962). It is also possible that the slow rate of the utilization of erucic acid during germination was responsible for its accumulation in the cotyledon portion.

The triglyceride fraction in the cotyledon indicated a similar increase of erucic acid incidental to germination. As over 75 per cent of the triglyrides contain erucic acid as the major acid (40 per cent of the total fatty acids) and as three-fourths of the total fraction of triglyceride disappeared during germination (Table 3), the possibility is indeed remote that the erucic acid-containing triglycerides escape utilization during the process. Thus it seems that during germination, the triglycerides are broken down into glycerol and fatty acids, and the unsaturated fatty acids, except erucic acid, are used up as a source of energy. Consequently, the new types of triglyrides, which are formed, possess even a higher concentration of erucic acid than the ones present in the seed before germination. In the cotyledon, which is the more actively metabolizable tissue, there was a considerable decrease in the content of palmitic acid of the whole oil, indicating its preferential utilization by the growing tissue.

Lipid Changes in Linseed (Cotyledons)

A rapid decrease of oil and triglyceride content, with a concomitant increase in the concentration of free fatty acids, was observed between 0-18 hours of germination (Table 2). After this period, up to 90 hours after germination, there was no increase in the concentration of partial glycerides and free fatty acids. The composition of the oil at any stage of germination w[illegible] determined by the balance of two competing processes, i.e. there is hydrolysis of triglycerides and sterol esters into free sterols and free fatty acids and the conversion of free fatty acids into carbohydrates for meeting the energy requirements of the growing organs. The above observation indicates that in the initial stages of germination (0-18 hours), a breakdown of sterol esters and triglycerides had taken place, leading to the accumulation

TABLE 3

The composition of fatty acids of *tarandra* oil during germination under aerobic conditions

Acid type	Days after germination								
	0	1	2	3	4	5	6	7	8
(a) *Whole seedlings (var. Local)*									
16:0	14.3		6.3		4.5		5.2		5.2
18:0	1.4		0.6		1.4		0.4		0.8
18:1	15.4		16.8		21.0		20.2		26.6
18:2	8.7		10.2		12.9		9.2		12.3
18:3	17.2		18.4		24.7		21.3		24.4
22:1	43.0		47.7		35.5		44.7		38.7
(b) *Cotyledons (var. Selection A)*									
16:0		9.8	9.0	9.4	10.5	8.7	8.3	9.9	10.1
18:0		1.4	1.2	1.0	0.2	1.2	0.1	0.3	0.9
18:1		22.9	17.0	16.4	13.5	12.1	11.7	14.5	13.6
18:2		12.8	11.2	12.2	8.4	7.4	7.1	8.6	9.2
18:3		21.7	24.6	22.0	24.6	20.6	16.9	11.6	12.9
22:1		32.0	37.0	39.0	42.8	50.0	55.9	55.1	53.3
(c) *Hypocotyls (var. Selection A)*									
16:0			7.8	8.2	7.4	14.8	22.6	34.2	39.3
18:0			0.8	1.0	0.4	3.0	2.3	Traces—	
18:1			11.2	10.2	10.9	5.3	7.1	8.1	10.6
18:2			10.4	11.8	10.0	10.8	16.2	13.4	11.6
18:3			31.2	29.2	30.8	25.4	9.6	16.0	11.5
22:1			38.4	39.6	40.5	40.7	42.2	28.3	27.0
(d) *Triglyceride fraction (cotyledon oil)*									
16:0	9.8		5.7		3.0		6.4		3.9
18:0	2.1		0.8		1.5		1.6		0.7
18:1	12.3		15.9		14.0		11.7		8.1
18:2	10.6		9.3		8.0		6.4		7.4
18:3	22.1		26.7		23.3		18.0		16.8
22:1	43.1		41.6		50.2		55.9		63.1

TABLE 4

The composition of the fatty acids of linseed oil and its triglyceride (variety K_2) during germination under unaerobic conditions (percentage basis)

Particulars acid type	Hours after germination					
	0	18	36	54	72	90
(a) *Total oil*						
16:0	10.5	11.4	9.8	9.9	10.8	9.2
18:0	4.2	3.8	2.6	4.6	5.2	4.6
18:1	27.6	28.4	26.4	23.8	21.8	23.2
18:2	14.2	16.2	15.8	18.2	14.9	16.8
18:3	43.5	40.2	45.4	13.5	47.5	46.2
(b) *Triglycerides*						
16:1		12.8	12.6	13.4	11.8	9.6
18:0		3.6	3.4	3.9	4.3	2.6
18:1		29.4	30.2	26.0	28.0	29.0
18:2		15.0	10.4	15.5	17.9	19.6
18:3		39.2	43.4	41.2	38.0	39.2

of free fatty acids and free sterols. The free fatty acids, consequently, were converted into carbohydrates as a source of energy for the germinating seeds (18-72 hours). Between 70 and 90 hours of germination, the accumulation of free fatty acids in the cotyledons was due to the death of the protoplasm. Zimmerman & Klosterman (1965) have reported that under incubation conditions at high temperatures and under a limited supply of oxygen, the metabolism of the lipids will be enhanced.

The composition of the fatty acids of linseed oil (var. K_2) and its triglycerides (Table 4) shows that there was a similar type of metabolism of fat and its unsaturated fatty acids throughout the germination period. Similar observations were recorded by Zimmerman & Klosterman (1965) from their studies on germinating flax seeds.

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